

DNA purification by centrifugation

- Dissolving the gel slice
 - Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5mL microcentrifuge tube
 - Add 10 μ L membrane binding solution per 10mg of gel slice. Vortex and incubate at 50-65°C until gel slice is completely dissolved
- Processing PCR amplifications
 - Add an equal volume of membrane binding solution to the PCR amplification
- Binding of DNA:
 - Insert SV minicolumn into collection tube
 - Transfer dissolved gel mixture or prepared PCR product to the minicolumn assembly
 - Incubate at room temperature for 1min
 - Centrifugate at 16,000g for 1min
 - Discard flowthrough and reinsert multicolumn into collection tube
- Washing:
 - Add 700 μ L membrane wash solution (ethanol added)
 - Centrifugate at 16,000g for 1min
 - Discard flowthrough and reinsert multicolumn into collection tube
 - Repeat step before with 500 μ L membrane wash solution
 - Centrifugate at 16,000g for 5min
 - Empty the collection tube and recentrifugate the column assembly for 1min with the microcentrifuge lid open to allow evaporation of any residual ethanol
- Elution:
 - Carefully transfer minicolumn to a clean 1.5mL microcentrifuge tube
 - Add 15 μ L of nuclease-free water to the mini column
 - Incubate at 60°C for 5min
 - Centrifugate at 16,000g for 1min
 - Repeat this step
 - Discard minicolumn and store DNA at 4°C or -20°C

